

Appl. No. 09/840,762
Amtdt. dated August 27, 2004
Amendment After Allowance for Sequence Listing

PATENT

Amendments to the Specification:

Please replace the paragraph beginning at page 18, line 4, with the following:

--DNA Hybridization Method. Hybridization probes were prepared at the second and near the third regions shown to be conserved between *Curvularia* and *Ascophyllum* vanadium peroxidase active sites by Messerschmidt, *et al.*, *PNAS*, 93:392-396 (1996). Hybridization probes of 51 base pairs were designed with Oligo 5.0 Primer Analysis Software (National Biochemicals, Plymouth, MN), synthesized by Anagen (Palo Alto), and digoxigenin-labeled at the 5' end with the Genius system (BMB Biochemicals, Durham, NC). The sequence of the probe for the second conserved site was:

CCAACGCACCCTCGTACCCGTCTGGCCACGCTACCCAAAACGGAGCATT (SEQ ID NO:3).

The sequence of the probe for the third conserved site was:

CCGTACGAACACTTCACCAGGAGCTGATGACTTCGCCGAGGAATCCACCT (SEQ ID NO:4).--

Please replace the paragraph beginning at page 19, line 14, with the following:

-- The following Fucus peroxidase LIC primers were designed with Oligo software (National Biosciences, Inc., Plymouth, MN) and pET-32 LIC sequences necessary for ~~incorporated incorporation~~ into the vector ~~(normal font)~~. Primers for the 5' end were:

GACGACGACAAGATGCTTGCCATGCAGCGGACA (SEQ ID NO:5) (34 bp) for the full length construct, GACGACGACAAGATGGCGCCGAATAGAAGGGACAA (SEQ ID NO:6) (35 bp) for the mid length construct, and

GACGACGACAAGATGCTCTCCGAGCGACCTTC (SEQ ID NO:7) (33 bp) for the short construct. One 3'-primer, GAGGAGAAGCCGGTTGCACTAAGCCTGGCAGT (SEQ ID

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NO:8) (33 bp) was used for all three constructs. PCR was carried out for 30 cycles of 3 min at 94°, 1.5 min at 55° C and 2.3 min at 72° C, in 7 mM MgSO4 for the full length construct and 4 mM MgSO4 for the two truncated constructs. The PCR products were electrophoresed in 1.5% agarose and stained with ethidium bromide. DNA was extracted from the excised bands in GenElute minus EtBr spincolumns (Supelco, Bellefonte, PA) and precipitated with ethanol.—

Please replace the paragraph beginning at page 28, line 1 with the following:

-- The *Fucus* sequence contains three conserved vanadium-binding regions (Messerschmidt *et al.*). The three conserved vanadium-binding regions are as follows: (1) amino acids 452-473 -AQRASCYQKWQVHRFARPEALG (SEQ ID NO:9); (2) amino acids 528-546 -PTIIPSYPSPGHATQNGAFAT (SEQ ID NO:10) and (3) amino acids 591-609 NKLAVNVAFGRQMLGIHYRFD (SEQ ID NO:11). -In the three conserved vanadium-binding regions the *Fucus* and *Ascophyllum* amino acid sequences differ only at two locations in the first conserved region (alanine at *Fucus* 455 substituted for serine at *Ascophyllum* 19, and cysteine at *Fucus* 457 substituted for tryptophan at *Ascophyllum* 21). These two amino acid differences are therefore likely to be related to the greater specific activity of the *Fucus* enzyme, as are other amino acid sequence differences in the catalytic frame (amino acids *Fucus* 441-636). A major difference between the *Fucus*, *Ascophyllum* and *Corallina* algal bromoperoxidases and the fungal chloroperoxidases and various phosphatases is the additional basic amino acids in the first conserved domain of the bromoperoxidases, histidine at *Fucus* 464 and leucine at *Fucus* 472 for the brown algal enzymes, with threonine instead of leucine for the *Corallina* enzyme. These additional amino acids in the first conserved region are likely to be related to the greater activity of the bromoperoxidases with bromide, which is larger than the chloride ion.—

Please cancel the present "SEQUENCE LISTING", pages 30-38, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 9, at the end of the application.